






Leptospira Antibody IgG GENLISA™ ELISA

REF : KBD950

Ver 1.0

IVD

Enzyme Immunoassay for the Qualitative Determination of Leptospira Antibody IgG in human serum and plasma.

| | | | |
|---|-----------------------------|---|--------------------------------|
| IVD | For In-vitro Diagnostic Use | REF | Catalog Number |
|  | Store At | LOT | Batch Code |
|  | Manufactured By |  | Biological Risk |
|  | Expiry Date |  | Consult Operating Instructions |

For In-vitro Diagnostic use only. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product. Any use of this product other than the permitted use without the express written authorization of KRISHGEN Pudgala LLP is strictly prohibited.

REF KBD950



96 tests

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Introduction:

Leptospirosis is considered an emerging zoonotic disease of global importance and is found in both urban and rural areas of affected countries. It is caused by the genus *Leptospira*, which are motile spirochaetes of which there are 16 genom-species as delineated by DNA–DNA hybridization. Transmission to humans occurs through penetration of the organism into the blood stream via cuts, skin abrasions or mucus membranes. Infection may range in presentation from subclinical to severe. Onset is usually sudden, with symptoms including headache, fever, muscle pains, conjunctival infection, meningitis and abdominal pains. Severe complications such as hepato-renal failure and central nervous system involvement may arise. It is also known as 'Weil's syndrome.'

Intended Use:

The Leptospira Antibody IgG GENLISA™ ELISA is intended for the qualitative determination of IgG class antibodies in human serum and plasma.

Principle:

Leptospira Antibody IgG GENLISA™ ELISA is an indirect enzyme linked immunosorbent assay for qualitative determination of IgG antibody present in the human serum and plasma. Antigens are pre-coated onto microwells. Samples/Controls are pipetted into microwells and Leptospira antibody present in sample binds to the antigen coated on the wells. Enzyme conjugate is pipetted and incubated to form an immune complex. After washing microwells in order to remove any non-specific binding, the substrate solution (A and B) is added to microwells and color develops proportionally to the amount of Leptospira Antibody present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Microtiter Coated Plate (8x12 wells) - 1 no
2. Negative Control - 2 ml
3. Positive Control - 2 ml
4. Cut-Off Control - 3 ml
5. Enzyme Conjugate - 20 ml
6. Wash Buffer (40X) - 20 ml
7. Sample Diluent - 100 ml
8. TMB Substrate - 12 ml
9. Stop Solution - 12 ml
10. Instruction Manual.

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Store main kit components at recommended storage temperature indicated on the component label.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions.
3. The Substrate is light-sensitive and should be protected from direct sunlight or UV sources.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

**Specimen Collection and Handling:**

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, recentrifuge.

Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, recentrifuge.

Sample Dilution:

To make 1:100 Dilution, dilute 10 ul Sample + 1 ml Sample Diluent.

Reagent Preparation:

1. Wash Buffer (1X) Dilution: To make Wash Buffer (1X), add 2.5 ml of Wash Buffer (40X) to 97.5 ml of DI water. This is the working solution.
2. Allow all components to reach RT (Room Temperature) prior to use in the assay.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.
2. Add **100 ul Control/Diluted Sample** in appropriate wells.
3. Seal the plate and Incubate at 37°C for 60 minutes.
4. Aspirate and wash plate 5 times with **(1X) Wash Buffer** and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly.
5. Add **100 ul of Enzyme Conjugate** to each well except blank well. Incubate at 37°C for 30 minutes.
6. Repeat the Aspiration/ Wash Step as mentioned in step 4.
3. Add **100 ul of TMB Substrate** into each well.
4. Incubate at 37°C for 10 minutes.
5. Add **100 ul of Stop Solution**. Read result with an ELISA reader at 450 nm within 15 minutes of stopping the reaction.

Criteria of Validation:

| | |
|-----------------------------|---------------------------|
| Blank | O.D < 0.100 |
| Negative Control | O.D < 0.200 and < Cut-Off |
| Cut-Off Control (CO) | O.D between 0.150 – 1.300 |
| Positive Control | O.D > Cut-Off |

Calculation:

Determine the Mean Absorbance (net of Blank) for each set of duplicate Controls and Samples.

$$O.D_{\text{Mean Cut-off Control (CO)}} = O.D_{\text{Cut-Off Control 1}} + O.D_{\text{Cut-Off Control 2}} / 2.$$

Results:

$$\frac{O.D_{\text{Mean Sample}} \times 10}{CO} = \text{Krishgen Units} = U$$

Interpretation of Results:

| | | |
|-----------------------|----------|---|
| Cut-Off Value | 10 U | |
| Equivocal | 9 – 11 U | Antibodies against the specific pathogen can not be detected clearly. |
| Negative Value | < 9 U | No antibodies present against specific pathogen. |
| Positive Value | > 11 U | Antibodies against specific pathogen are present. |

State Of Infection:

| Serology | Significance |
|----------|--|
| IgM | Primary Antibody Response High IgM titer with Low IgG titer: Current or recent Infection. Persisting IgM: Rare. |
| IgG | Secondary Antibody Response. May persist for several years. High IgG titer with Low IgM titer: Indicates Past Infection. |

Reference Values:

It is recommended that each laboratory establishes its own normal and pathological reference ranges, as usually done for other diagnostic parameters, too.

Limitations of Method:

Any clinical diagnosis should not be based on the results of in vitro diagnostic methods alone. Physicians are supposed to consider all clinical and laboratory findings possible to state a diagnosis.

Performance Characteristics:**Sensitivity:**

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100.0% (95% confidence interval: 93.4% - 100.0%).

Specificity:

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 97.37% (95% confidence interval: 86.19% - 99.93%).

Precision:

Intra-Assay

| Intra-assay | n | Mean (E) | CV (%) |
|-------------|----|----------|--------|
| 1 | 24 | 0.470 | 2.95 |
| 2 | 24 | 0.782 | 6.50 |
| 3 | 24 | 0.460 | 3.49 |

Inter Assay

| Inter-assay | n | Mean (U) | CV (%) |
|-------------|----|--------------|--------|
| 1 | 12 | 19.60 | 4.87 |
| 2 | 12 | 12.72 | 9.64 |
| 3 | 12 | 3.99 | 4.85 |

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only.** Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
 - Do not smoke, eat or drink while handling kit material
 - Always use protective gloves
 - Never pipette material by mouth
 - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

**LIMITED WARRANTY**

Krishgen Pudgala LLP does not warrant against damages or defects arising in shipping or handling, or out of accident or improper or abnormal use of the product; against defects in products or components not manufactured by Krishgen Pudgala LLP, or against damages resulting from such non-Krishgen Pudgala LLP made products or components. Krishgen Pudgala LLP passes on to customer the warranty it received (if any) from the maker thereof of such non-Krishgen made products or components. This warranty also does not apply to product to which changes or modifications have been made or attempted by persons other than pursuant to written authorization by Krishgen Pudgala LLP.

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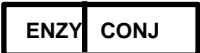








Regulatory Status:

| | |
|------------------|--------|
| CE Marked | Europe |
| FDA registered | USA |
| CDSCO registered | India |

SCHEMATIC ASSAY PROCEDURE

| | |
|----|---|
| 1 | All reagents should be allowed to reach room temperature before use. |
| 2 | Add 100 ul Control/Diluted Sample in appropriate wells |
| 3 | Seal the plate and Incubate at 37°C for 60 minutes. |
| 4 | Aspirate and wash plate 5 times with (1X) Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step. All the washes should be performed similarly. |
| 5 | Add 100 ul of Enzyme Conjugate to each well except blank well. |
| 6 | Incubate at 37°C for 30 minutes. |
| 7 | Repeat the Aspiration / Wash Step as mentioned in step 4. |
| 8 | Add 100 ul of TMB Substrate into each well. |
| 9 | Incubate at 37°C for 10 minutes. |
| 10 | Add 100 ul of Stop Solution . Read result with an ELISA reader at 450 nm within 15 minutes stopping the reaction. |

SYMBOLS KEY

| | |
|---|--------------------------------------|
|  | Coated Microtiter Plate (8x12 wells) |
|  | Control |
|  | Enzyme Conjugate |
|  | Sample Diluent |
|  | (40X) Wash Buffer |
|  | TMB Substrate |
|  | Stop Solution |
|  | Consult Instructions for Use |
|  | Catalog Number |
|  | Expiration Date |
|  | Storage Temperature |